In the Specification:

Replace the paragraph at page 4, lines 22-29 of the specification with the following paragraph,

B2

(C) Amino acid sequence alignment of human Ire1p (H.s.) (SEQ ID NO:2), *S. cerevisiae Ire1p (S.c.)* (SEQ ID NO:4) *and its putative homologous protein from C. elegans (C.e.)* (SEQ ID NO:3). Open boxes indicate the identical sequence. Shaded boxes indicate conserved residues. Dashes represent gaps between residues in order to obtain maximum matching. Numbers are the position of the last amino acid. ∇, potential signal peptide cleavage site; ∘, invariant residues in protein kinase domain; *, indicates the invariant Lys599 residue in kinase subdomain II. The glutamine rich cluster (I) and the serine rich cluster (II) in the linker region are also identified.

Replace the paragraph at page 7, lines 13-15 of the specification with the following paragraph,

B3

Figures 8A-8B. The nucleotide sequence of *hIRE1* of the present invention (SEQ ID NO:1). The sequence information has been submitted to GenBank under accession no. AF059198 and is expressly incorporated by reference.

Replace the paragraph at page 7, lines 16-18 of the specification with the following paragraph,

B4

Figure 9. The amino acid sequence of hIre1p of the present invention (SEQ ID NO:2). See also, Tirasophon, W. et al., *Genes Dev.* 12:1812-1824 (1998), expressly incorporated by reference.

Replace the paragraph at page 7, lines 20-23 of the specification with the following paragraph,

B5

A novel mammalian bifunctional protein kinase/endoribonuclease referred to herein as hIre1p, is provided. Also provided is a novel cDNA (*hIRE1*) encoding hIre1p (SEQ ID NO:1). The isolated cDNA is about 3.6 kbp long with an open reading frame extending from nucleotide 97 to 3030, encoding the novel mammalian protein, hIre1p.

Replace the paragraph at page 7, lines 24-29 of the specification with the following paragraph,

B6

The nucleic acid sequence of the cDNA encoding Ire1p and its deduced amino acid sequence are set forth in Figures 8A-8B (SEQ ID NO:1) and Figure 9 (SEQ ID NO:2), respectively. In a preferred embodiment, the isolated nucleic acid molecule of the invention comprises the nucleotide sequence of Figures 8A and 8B (SEQ ID NO:1), or homologues therefore. In another preferred embodiment, the isolated and purified polypeptide of the invention comprises the amino acid sequence of Figure 9 (SEQ ID NO:2), as well as biological equivalents.

Replace the paragraph at page 9, lines 8-28 of the specification with the following paragraph,

B7

In another aspect, DNA sequence information provided by the present invention allows for the preparation of relatively short DNA (or RNA) sequences or probes that are identical to or hybridize to the nucleotide sequence disclosed herein. Nucleic acid probes (also referred to as oligonucleotide probes) of an appropriate length are prepared based on a consideration of the nucleotide sequence of Figures 8A and 8B (SEQ ID NO:1). The probes can be used in a variety of assays appreciated by those skilled in the art, for detecting the presence of complementary sequences in a given sample. The probes may be useful in research, prognostic and diagnostic applications. For example, the probes may be used to detect homologus nucleotide sequences, *e.g.*, the human homolog. The design of the probe should preferably follow these parameters:

a) it should be designed to an area of the sequence which has the fewest ambiguous bases ("N's"), if any; and

b) it should be designed to have a T_m of approximately 80° C (assuming 2 degrees for each A or T and 4 degrees for each G or C). The oligonucleotide should preferably be labeled with γ-^{32}P ATP (specific activity 6000 Ci/mole) and T4 polynucleotide kinase using commonly employed techniques for labeling oligonucleotides. Other labeling techniques can also be used. Unincorporated label should preferably be removed by gel filtration chromatography or other established methods. The amount of radioactivity incorporated into the probe should be quantitated by measurement in a scintillation counter. Preferably, specific activity of the resulting probe should be approximately 4e+6 dpm/mole.

Replace the paragraph at page 9, line 29 through page 10, line 10 of the specification with the following paragraph,

B8

A further preferred nucleic acid sequence employed for hybridization studies or assays includes probe molecules that are complementary to at least a 10 to 70 or so long nucleotide stretch of the polynucleotide sequence shown in Figures 8A and 8B (SEQ ID NO:1). A size of at least 10 nucleotides in length helps to ensure that the fragment will be of sufficient length to form a duplex molecule that is both stable and selective. Molecules having complementary sequences over stretches greater than 10 bases in length are generally preferred in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. It will be appreciated that nucleic acid molecules having gene-complementary stretches of 25 to 40 nucleotides, 55 to 70 nucleotides, or even longer where desired, may be preferred. Such fragments can be readily prepared by, for example, directly synthesizing the fragment by chemical means, by application of nucleic acid reproduction technology, such as the PCR technology of U.S. Patent 4,683,202, or by excising selected DNA fragments from recombinant plasmids containing appropriate inserts and suitable restriction enzyme sites. In certain embodiments, it is also advantageous to use oligonucleotide primers. The sequence of such primers is designed using the polynucleotide of the present invention and is used with PCR technology.

Replace the paragraph at page 15, line 31 through page 16, line 20 of the specification with the following paragraph,

B9

Isolation of complementary DNA encoding human Ire1p. To screen for a human homologue of *S. cerevisiae IRE*1, degenerate oligonucleotide primers were designed from the amino acid sequence (ISDFGLCK) (SEQ ID NO:5) in the kinase subdomain VII of *S. cerevisiae IRE1* that was also conserved in a putative *C. elegans IRE1* identified in the genbank, but was not present in other protein Ser/Thr protein kinases. The oligonucleotide was used in combination with a ëgt 10 specific primer to amplify DNA fragments from a human fetal liver cDNA library. RH3 was isolated as a candidate clone containing a 270 bp PCR product, that encoded for a portion of the catalytic domain of a novel human Ser/Thr protein kinase. The clone was used as a probe

to screen for overlapping clones from a human fetal liver cDNA library (Fig. 1A). A 3.5 kb cDNA was assembled from overlapping clones that has a single open reading frame encoding 977 amino acid residues with a predicted molecular mass of 110 kDa (Fig. 1C). One clone had a 106 bp putative 5' untranslated region that did not contain either an ATG codon or an in frame termination codon upstream of the ATG codon having a favorable sequence context (CGCCATGCC) (SEQ ID NO:6) to serve as an initiation codon. Kozak, M., *Nucl. Acids Res.* 15:8125-8248 (1987). In addition, immediately following the putative initiation codon was a sequence of residues that are predicted to serve as a signal peptide, having positively charged residues at the extreme N-terminus followed by a core of hydrophobic residues and then turn inducing residues (Pro and Gly). Nielsen, H. et al., *Protein Eng.* 10:1-6 (1997). We predict that signal cleavage occurs after Gly18. Finally, one clone (13-1) was identified that contained a 3' untranslated region of 598 bp that contained multiple translation termination codons in all reading frames, but did not contain a conserved polyadenylation signal, suggesting additional sequence exists within the 3' untranslated region of the mRNA. On the basis of these observations, it is believed the intact coding region for this putative kinase is cloned.

Replace the paragraph at page 16, line 29 through page 17, line 11 of the specification with the following paragraph,

Searches of the protein sequence database suggested that the carboxy terminal half of the protein could be divided into 3 domains: a linker region, the putative Ser/Thr protein kinase domain, and an RNase L-like domain homologous to 2'-5' oligo A-dependent ribonuclease (Fig. 1B). The linker region is unique to the human protein and contains two subdomains rich in glutamine and serine residues, respectively. In contrast, the kinase domain displays high homology with both that of *S. cerevisiae* Ire1p and its putative counterpart from *C. elegans*, having conserved all 12 invariant residues that are present within the protein kinase superfamily (Fig. 1C). Hanks, S.K. et al., *FASEB J.* 9:576-596 (1995). The similarity among these three proteins also extends to the very carboxy terminal RNase L domain (Fig. 1C). Zhou A. et al., *Cell* 72:753-765 (1993). The cytoplasmic domain of hIre1p, as well as its putative counterpart from *C. elegans*, do differ from yeast Ire1p in that they do not contain a potential nuclear localization signal and also lack an insertion of 30 hydrophilic amino acids between conserved kinase subdomains VIII and IX. Mori, K. et al., *Cell* 74:743-756 (1993). In contrast, the amino terminal half of the

B10 Cont'd

protein was less conserved, with the *C. elegans* protein (SEQ ID NO:3) and displayed no significant similarity with *S. cerevisiae* Ire1p (SEQ ID NO:4). Based on the homologies within the cytoplasmic domain, this putative human protein is referred to as human Ire1p (hIre1p).

Replace the paragraph at page 22, line 17, through page 23, line 1 of the specification with the following paragraph,

B11

Cloning of human IRE1 cDNA. A degenerate antisense oligonucleotide [5' (TC)TT (AG)CT IT(AG) ICC (AG)AA (AG)TC IG(AT) IAT 3'](SEQ ID NO:7) was designed from the conserved amino acid sequence in kinase subdomain VII (ISDFGLCK) (SEQ ID NO:8) between *S. cerevisiae IRE1/ERN1* and its putative homologue from *C. elegans*. Inosine was incorporated into positions to minimize degeneracy and improve stability upon hybridization (Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning: A laboratory manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory press)). This primer and a ëgt 10 specific primer were used to amplify sequences from a human fetal liver cDNA library (Clontech). Total PCR products were ligated into the TA cloning vector (Invitrogen) and transformed into *E. coli DH5á*. A candidate clone, RH3, that showed highest homology to the yeast *IRE1* and its counterpart gene in *C. elegans* was subsequently used to screen the ëgt 10 human fetal liver cDNA library by standard procedures (Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular cloning: A laboratory manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory press)). The 5' end of the *hIRE1* (F14) was obtained by 5' rapid amplification of cDNA ends (RACE) PCR (Bethesda Research Labs) using template RNA isolated from the human hepatoma cell line HepG2. Each cDNA fragment was subcloned into pBluescript II SK(-) plasmid (Stratagene) at the *EcoR* I site. All cDNA fragments were sequenced from both directions using the dideoxynucleotide sequencing method (Sequenase, Amersham).

Replace the paragraph at page 23, lines 2-16 of the specification with the following paragraph,

B12

pBluescript -13-1 is a recombinant plasmid containing the largest open reading frame of hIre1p but lacking the 5' end fragment. To assemble the full length*hIRE1* cDNA, the 0.3 kb PCR product including the initiator methionine was amplified from pBluescript-

F14 using two primers: 1058G (5' GCT CTA GAA CCA TGC CGG CCC GGCGGC T 3') (SEQ ID NO:9) and 865G (5' AGG CTG CCA TCA TTA GGA TCT 3') (SEQ ID NO:10) and Vent DNA polymerase (New England Biolabs). The 0.3 kb PCR product was introduced into clone 17-1 by overlap-extension PCR using two primers: 1058G and 9241B (5' CAT TGA TGT GCA TCA CCT TCC TC 3') (SEQ ID NO:11) to yield a 0.7 kb PCR product. The 0.7 kb fragment was digested with *Xba* I, located upstream to the first ATG introduced by PCR, and *BamH* I. The fragment was ligated to pBluescript-17-1 at the same restriction endonuclease sites to yield pBluescript-17-1/5'. The 0.9kb *Xba* I/*Sac* II fragment from pBluescript-17-1/5' was ligated to pBluescript -13-1 at the same sites to yield pBluescript -*hIRE1*. The 3.5 kb *Xba* I/*EcoR* I *hIRE1* cDNA was subcloned into the *Xba*I site of the mammalian expression vector, pED (pEDΔC) (Kaufman, R.J. et al., *Nucl. Acids Res.* 19:4485-4490 (1991)) to yield pED-*hIRE1*.

B12 Cont'd

Replace the paragraph at page 23, line 23, through page 24, line 2 of the specification with the following paragraph,

B13

Antibody production. The 1.6 kb cDNA encoding the entire cytoplasmic domain of hIre1p (amino acid residues 460 to 977) was generated by PCR amplification using primer 168G (5' CGG AAT TCA TCA CCT ATC CCC TGA GCA TG 3'), (SEQ ID NO:12),169G (5' CGG AAT TCT CAG AGG GCG TCT GGA GTC A 3')(SEQ ID NO:13) and Vent DNA polymerase (New England Biolabs). In order to make GST-hIre1p fusion protein, the PCR product was inserted in frame into pGEX-1λT (Pharmacia) at the *EcoR* I site and then transformed into *Escherichia coli DH5á*. The fusion protein was produced and purified as described by Frangioni and Neel (*Cell* 57:1069-1072 (1993)) except that the induction was performed at 30°C. The purified GST-cytoplasmic hIre1p fusion protein was repeatedly injected into mice as described by Harlow and Lane (1988). Sera collected from tail bleed was used for determining the titer by Western blot analysis. When optimal titer was obtained, the mice were injected with the sarcoma cell line S180 to induce ascites fluid that was directly used. Harlow, E. and Lane, D. 1988. Antibodies: A laboratory manual (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory press).